REMARKS/ARGUMENTS

In the present Office Action, claims 9-17 and 20-21 were withdrawn from further consideration as being directed to a non-elected invention. Election of claim 1-8 and 18-19 was acknowledged. By this Amendment, claims 1 and 18 are amended, and claim 19 is canceled.

In view of the above amendments and the following remarks, the Examiner is respectfully requested to allow claims 1 -8 and 18, claims 9 – 17, 20 and 21 having been withdrawn, and claim 19 having been canceled, comprising all of the claims of the original application. Upon allowance of the claims under consideration, the Examiner is authorized to cancel the withdrawn claims and is requested to pass this application to issue.

Before embarking on responding to each of the issues raised in the Official Action, a general statement is believed appropriate. It is black letter law that a patent application is written to those of ordinary skill in the art. Therefore, applicants need not provide the obvious or burden the claims with materials that are not essential to the patentability of the invention. Rather, the specification provides guidelines as to what is intended and, extrapolating from the working exemplification, those of ordinary skill in the art would have no difficulty in practicing the invention and understanding the scope of the claims. This statement is being made as it is submitted that some of the rejections of the claims under §112 would appear to suggest that the application is directed to laymen who have no skill in the relevant art. Furthermore, the relevant art of binding assays is now well over 50 years old, the Nobel Prize having been granted in 1977 to Rosalyn Yalow for the discovery of radioimmunoassays and protein binding assays which preceded this discovery. The major step that the subject invention provides is a combination of reagents that are found to give sensitive assays in the presence of confusingly like compounds. It is noted that the claims in Sportsman et al. cited by the Examiner have many of the same alleged failings as the subject claims.

Turning now to the Detailed Action, the following remarks are set forth and responded to in the same order as presented in the Office Action.

Information Disclosure Statement (Office Action Paragraph 2)

While it would appear that the Official Action has captured all of the relevant literature, any additional literature would only be redundant. However, an IDS on PTO form 1449 accompanies this response in order to ensure that references, including those listed at pp. 2-3 of the specification, are properly considered. References from the Supplementary Examination Report by the EPO, received March 8, 2007, are listed as follows:

- Bosse Abstract, FASEB J. 16(4):p. A575 (2002);
- Rossi et al., "Monitoring protein-protein interactions in intact eukaryotic cells by β-galactosidase complementation," Proc. Nat. Acad. Sci. USA 94:8405-8410 (1997);
- Hirata et all., "Stereospecific Recognition of Inositol 1,4,5-triphosphate Analogs by the Phosphatase, Kinase, and Binding Proteins," J. Biol. Chem. 265(15):8404-8407 (1990);
- Riley et al., "Interactions of Inositol 1,4,5-Triphosphate (IP3) Receptors with Synthetic Poly(ethylene glycol)-linked Dimers of IP3 Suggest Close Spacing of the IP3 Binding Sites," J. Biol. Chem 277(43) (40290-40295 (already cited by the USPTO);
- Perkin Elmer product insert for Alphascreen Protocols, "Performing AlphaScreen tm IP3 Functional Assays," (c) 2002;
- EP 0992587; and
- US 5,252,707.

The citation of any reference in the present IDS does not constitute an admission that any cited document is prior art.

Claim Objections (Office Action Paragraph 3)

Claim 1 was objected to for language informalities. It was suggested that abbreviations be spelled out.

Applicants' Response

The informalities pointed out in the Official Action have been avoided by amendment. Claim 1 has been amended to be congruent with claim 9, an original claim.

Claim Rejections – 35 USC §112 (Office Action Paragraphs 4-13)

Claims 1-8 and 18 and 19 were rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. The Office Action contains the following paragraphs, responded to here:

> **7.** Claim 1 recites the phrase "a detectable label joined through a bond or linker at the 2-hydroxyl position". It is not clear whether the "2-hydroxyl position" is of the "inositol 1 ,4,5-triphosphate (IP3)" or other compound. If it is IP_3, a phrase "of said IP3" should be inserted after "2-hydroxyl position" in order to clearly define the point of linkage.
>
> **8.** Claim 1 lacks antecedent basis for the term "said binding protein" in lines 6-7.
>
> **9.** The recitation "sufficient time" in line 7 of claim 1 is indefinite. It is not clear how one can determine with clarity and accuracy the length of time that is sufficient for any IP_3 and said conjugate to bind to said binding proteins and a the length of time in a condition that is sufficient in one case, may not be sufficient for another. Applicant is advised to define the term "sufficient time".
>
> **10.** Claim 1 recites the phrase "detecting the bound and unbound label as a measure of the IP_3 present in the sample" in lines 9-10. The step should be re-written to clearly indicate how "detecting the bound or unbound label" is correlated with the "measuring of IP_3".
>
> **11.** Claim 3 recites the phrase "wherein said cellular lysate has been treated to block kinases and phosphatases". It is unclear cellular lysate is treated with "what" in order to block kinases and phospatases i.e. the process by which kinases and phosphatases in said cellular lysate is blocked is unclear.
>
> **12.** Claim 6 recites the phrase "wherein said binding protein is a fusion protein of up to about 1.5 kD amino acids". The nature and chemical structure of the fusion protein in unclear because it's not clear what sequence or portion of the IP_3R is fused to "what" amino acid sequence or protein or peptide sequence.
>
> **13.** The dependency of claim 18 and 19 should be corrected to properly make it dependent on an elected claim of 1-8 and should be re-written to include all the limitations of claim 1 or any intervening claims.

Applicants' Response

The rejection of paragraph **7** of the Official Action has been avoided by amendment.

The rejection of paragraph **8** of the Official Action has been avoided by amendment.

The rejection of paragraph **9** of the Official Action is traversed. The claim recites "for sufficient time for complex formation of IP_3 and said conjugate with said binding protein." Therefore, a clear measure is provided for determining for how long the incubation should be performed. Those of skill in the art would know that when there is a detectable signal with a known amount of the analyte, that time may be used for the assay. Some research is permitted, so long as it is routine. It is routine in the assay field to use known amounts of an analyte and carry out the assay, using different times for the determination. As the application shows, reasonable times may be used in determining 1,4,5-triphosphate inositol with the subject protocol. See paragraphs[00070] – [00075] of the specification.

The rejection of paragraph **10** of the Official Action is in part avoided and in part traversed. Applicants' attorney has amended the claim to further indicate what is intended by measuring the bound or unbound label. However, enzyme fragment complementation assays are hoary with age and well known. Similarly, fluorescent assays are well known. The subject invention is not the discovery to these assays but their application to a particular context. As such, those of ordinary skill in the art would have no problem in determining the breadth of the claim and the intent of the language.

The rejection of paragraph **11** of the Official Action is traversed. First, the Examiner's attention is directed to paragraphs [00048] and [00073] of the specification. Blocking phosphatase and kinase enzymes is not a novel procedure for measuring phosporylated analytes. There are 4574 entries in Entrez-PubMed under a search for "phosphatase and kinase inhibition." In view of the importance of phosphorylation and dephosphorylation in cellular activity, numerous ways have been developed to prevent changes in the level of phosphorylation while one is measuring the degree of phosphorylation. This step is a conventional step commonly used in such assays and should not require any more in the claim than the statement that inhibition is employed.

The rejection of paragraph **12** of the Official Action is traversed. Applicants do not rely on the fact of a fusion protein for patentability. Fusion proteins are well known and their preparation is well within the skill of the art. Applicants refer to fusion proteins in the

specification at paragraph [00042]. Such protein is exemplified, since the subject binding protein is a fusion protein with GST. Other examples of fusion proteins are given, such as fusion with GFP. The choice of the fusion protein is one of interest or convenience and does not change the nature of the invention. The important factor is that one should not be able to avoid the subject claims by making a fusion protein of the binding protein and argue that it is not longer the same as the binding protein. Since a basis of the invention is the binding protein, modifications that do not interfere with the assay should be permitted to be claimed. If someone wishes to check whether a particular fusion protein is operable, there are guidelines in the subject application and such fusion protein is at the periphery of the invention.

The rejection of paragraph **13** has been avoided by amendment.

Claim Rejections --claims 1-3 and 5-8 --under 35 USC § 112, first paragraph, as not enabled (Office Action Paragraphs 14, 15)

Paragraph **15** states in part:

> Claims 1-3 and 5-8 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for truncated extracellular portion of mouse Type1 $1P_3R$ comprising at least amino acids sequence of 226-578, do not reasonably provide enablement for all any truncated portion (sequence) of IP_3R as binding protein which would have an affinity of at least about 200 times the affinity for IP_3 than that of intact IP_3R for IP_3.

Applicants' Response

The rejection of paragraph **15** of the Official Action is traversed. The subject invention is not the binding protein. The claims are for a method to determine 1,4,5-triphosphate inositol. There is no rejection that the subject methodology is not useful or operable. Rather, the Official Action indicates that having one species of the binding protein is not sufficient to support any binding protein having the characteristics set forth in claims 1 – 3 and 5 – 8. However, if anyone wishes to perform the assay, applicants have provided a satisfactory method. Should anyone find another binding protein having the required characteristics and uses such protein in the subject method, such use should be an infringement. What applicants have brought to the public is a

method of measuring 1,4,5-triphosphate inositol. Applicants are not required to provide alternative compositions for performing their method. All that should be required is that they define the characteristics of one of the reagents and exemplify such reagent. The public is then assured of being able to assay for 1,4,5-triphosphate inositol and may use alternative reagents, should such reagents become available. The invention is the method of determining 1,4,5-triphosphate inositol, not the reagent *per se*. The specification does allow anyone to practice the claimed invention. It is not up to applicants to have to provide alternative reagents, when the reagent that is provided is adequate for the purpose of their invention and the claims require that the reagent have the characteristics set forth as equivalent to what has been provided.

Claim Rejections -1-4, 6-8 and 18-19 under 35 USC § 103(a) (Office Action Paragraphs 16, 17)

Claims 1-4, 6-8 and 18-19 were rejected as being unpatentable over Sportsman, et al (US 6,806,053 B1) in view of Iwasaki et al. (J. Biol. Chem. 2002) and Hirata et al. (J. Biol. Chem 1990). Claim 5 was rejected under 35 USC § 103(a) as being unpatentable over Sportsman et al., (US 6,806,053 B1) in view of Iwasaki et al. (J. Biol. Chem. 2002) and Hirata et al. (J. Biol. Chem 1990) as applied to claims 1-4 and 18-19 above and further in view of Henderson et al. (US 4,708,929).

The rejection is summarized from the Office Action as follows:

Sportsman et al. in a cell-signaling assay of inositos-phospholipid signaling pathway, disclose detection of intermediate 1,4, 5 IP_3 of the signaling pathway. The assay includes a tracer from the intermediate (i.e. tracer of 1,4, 5 IP_3) and a specific binding partner for 1,4,5 IP_3 (intermediate) and the tracer (e.g. labeled 1,4, 5 IP_3).

Iwasaki et al. disclose IP_3R antagonists that strongly and specifically bind to IP_3 (analyte).

Since specific binding partner for IP_3 is common and known in the art (Iwasaki et al.), it would have been obvious at the time of the invention to a person of ordinary skill in the art to include IP_3R receptor or truncated portion of the IP_3R as taught by Iwasaki et al in the assay method of Sportsman to effectively measure IP_3 in a sample with a reasonable expectation of success because specific binding partner for IP3 is envisaged in the method of Sportsman et al. ...

18. Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sportsman et al. (US 6,806,053 B 1), Iwasaki et al. (J. BioI. Chem. 2002) and Hirata et al. (J. Biol. Chem. 1990) as

applied to claims 1-4, 6-8 and 18-19 above and further in view of Henderson et al. (US 4708,929). ....Henderson et al. in a competitive assay for protein binding disclose labeling analyte with enzyme fragment (donor enzyme fragment e.g. enzyme donor of b- galactosidase) for detection by complementation with an enzyme acceptor that results in measurable enzyme activity (abstract and column 10, line 57 through column ,11, line 32). Henderson et al. also disclose that enzyme complementation is advantageous over other immunoassays employing fluorescent label as fluorescent label analyte require separation steps and are limited to small molecular weight analytes.

Applicants' Response

The most pertinent portion of the Sportsman et al. disclosure is as follows, taken from Example 14:

> "This example shows assays for components of the inositol-phospholipid signaling pathway, including associated G-proteins. Generally, the assays include luminescence polarization assays directed to intermediates of this pathway, such as 1,4,5 IP_3. The assays include a tracer form of the intermediate and a specific binding partner of the intermediate and tracer. The tracer may include a luminophore attached by a suitable chemistry to the intermediate (such as a fluorescein succinyl-labeled IP3). The binding partner may include an antibody that specifically binds to the intermediate and tracer. Assays may be performed as shown in FIG. 5, with the intermediate taking the place of the cyclic nucleotide. Assays for associated G-proteins may be performed as shown in FIG. 6. These assays may be used to measure the presence, concentration, and/or activity of intermediates, enzymes, and/or receptors involved, in this pathway, or they may be directed to associated tissues and responses, as indicated in the following table:
>
> Selected Hormone induced cellular Responses Medicated by G-Protein-linked Receptors Coupled to the Inositol-Phospholipid Signal Pathway

Target Tissue	Signaling Molecule	Major Response
Liver	Vasopressin	Glycogen breakdown
Pancreas	Acetylcholine	Amylase secretion
Smooth muscle	Acetylcholine	Contractions
Mast cells	Antigen	Histamine secretion
Blood platelets	Thrombin	Aggregation

As may be noted, there is no binding protein disclosed except for an allusion to an antibody. The example is an invitation for someone to provide an assay for IP3, not a disclosure of an assay. Sportsman et al. does indicate the general tools of the trade: a binding protein with

no indication of how one would form such antibody in view of the complicated nature of IP3, a tracer form of the intermediate with no suggestion of how that intermediate may be prepared in view of the complications associated with synthetic procedures for modifying IP3; and no indication of any problems associated with measuring IP3.

To get to the quick, both the binding protein and the derivative used by applicants were based on the literature. Applicants do not rely on the novelty of these reagents as a basis for patentability. Rather it was the bringing together of the reagents used by applicants and the development of a sensitive and specific assay for IP_3 that is the basis for patentability. Having accepted that it is not the discovery of the reagents that supports patentability, let us look at the prior art. Hirata is important for allegedly supporting the proposition that "... a series of 1,4,5-triphosphate (IP_3) analogs with substituents at 2 hydroxy position and disclose that such modification (substituents at 2-hydroxy position) do not substantially interfere with the affinity of IP_3 for IP_3 receptor." One turns to the graph of Fig. 6 for confirmation. It is noted that the radioactive IP_3 is present in slightly less than 1nM. The reaction being performed is a displacement, so that the rat cerebellum microsome is incubated with the radioactive IP_3 in the presence of the test compounds or non-radioactive IP_3 and incubated at ice temperature for 15 min. After filtration, the radioactivity of the filter is determined. It is somewhat puzzling that at equimolar concentration of the radioactive and non-radioactive IP_3, there is 90% of the radioactivity retained on the filter. In order that there only be 50% retention one needs a 10-fold greater concentration of the non-radioactive IP_3. Even at 30-fold greater concentration of the non-radioactive IP_3, 40% of the radioactivity is retained on the filter. The result appears to suggest the impossible result that the microsome can discriminate between the presence and absence of a tritium. How then is one to explain the results? Possibly, there is binding occurring that is not specific, so that the radioactivity binds to sites other than the receptor site. There may be other explanations, but there is clearly some discrepancy in the data. Since the authors are using a crude homogenate, one cannot be certain as to what is occurring.

In addition, when one studies the graph there are substantial differences in binding activity between the three test compounds. Using rough approximations, at 1nM, IP_3 reduces binding by about 15%, D-206 and D-209 are not reported. If one looks at 3nM concentration, IP_3 has reduced binding by about 25%, while with D-206 there is substantially no reduction and

D-209 is about 15% reduction. It is only at about 30 nM, 30 times the concentration of the tritiated IP_3 that the three test compounds have about the same inhibition. However, at this ratio, one cannot be certain as to whether one is looking at specific effects. Such major differences in binding affinity would create serious difficulties in developing an assay.

There is the further consideration that applicants are not using the rat receptor, but the mouse receptor and there is no certainty that the two receptors have the same spatial requirements for binding. The fact that they bind to the same ligand, does not mean that they would respond in the same way to a modification of the ligand. While it is appreciated that applicants' claims are not limited to mouse receptor, there are the further constraints that the receptor is truncated and has a 200-fold higher affinity. Only if it were shown that the rat receptor had these characteristics would it be appropriate to equate the two for the purposes of prior art.

There is the further consideration that when affinity increases, particularly where affinity increases 200-fold, one expects that the conformational requirements will be more stringent. Therefore, increased affinity is likely to lead to rejection of a modified ligand. One could not have predicted that the "sponge" receptor would accept any changes in the IP_3. However, as applicants have shown, the combination of the sponge receptor and the labeled IP_3, particularly when coupled with the improvements claimed in the dependent claims provides for a highly successful commercial assay. Accompanying this response are product inserts for the IP_3 assay. The enclosed Exhibit 1 relates to DiscovRx HitHunter™ Inositol (1, 4, 5) Trisphosphate Assay based on the claimed invention.

For any research director to perform his/her duties, programs that are selected should have some promise of success. In some instances, the outcome cannot be predicted. In order to justify the time and expense of carrying out the research to determine a commercially viable product, one must believe that there is the possibility of success. If the possibility of success becomes the standard for patentability, then commercial laboratories will be unable to apply for patents. Everything will be obvious under the repudiated standard of "obvious to try." It is submitted that the present modifications are not even obvious to try. The Official Action has put forward a case that does not even meet such rejected standard. Rather, because of the greatly

increased affinity of the binding protein, the expectation would be that modification of the IP_3, particularly with a bulky polypeptide would have reduced, if not negated, the binding affinity. That the opposite proved true may have been a hope prior to performing the research, but was hardly a reasonable expectation of success.

The rejection of paragraph **18** of the Official Action adding Henderson in relation to claim 5 is noted, but as already indicated applicants do not rely on the choice of the reagents as the basis for patentability. Therefore, for the reasons given previously, this rejection is also traversed.

Finally, it is noted that Riley is cited for disclosing a PEG linker at the 2-position of IP_3. With all due respect to the authors, they are not observing affinity, but rather avidity, since the IP_3 dimer is binding at two receptor positions. It should also be noted that the monomer IP_3 PEG conjugate has a 2-fold weaker binding affinity and PEG is unique in its characteristics.

Conclusion

Applicants request reconsideration and allowance of claims 1-8 and 18-19 for the reasons advanced above. It is believed that the present Amendment is fully responsive to the presently outstanding Office Action. For all of the above reasons in conjunction with the amendments, the application is considered in good and proper form for allowance and the Examiner is respectfully requested to allow the claims under consideration and pass this application to issue. As indicated previously, upon allowance of the claims under consideration, the Examiner is authorized to cancel the restricted claims. If the Examiner believes that prosecution could be expedited by a telephonic interview, the Examiner is requested to call Bertram Rowland, Reg. no. 20,015 at 650 344 4674.

Dated: 03/16/2007

Respectfully submitted,

PETERS VERNY, LLP

By _____

David J. Aston, Reg. No. 28,051
Tel.: (650) 324-1677
Fax: (650) 324-1678

STORAGE CONDITIONS

Upon receipt, store HitHunter IP_3 FP Assay - green as follows:

Condition	Temperature	Length of Time
Upon Arrival	– 80 ºC	Date on outer kit box
After thawing	4°C	2 weeks

Thaw reagents completely; Reagents should be allowed to equilibrate to ambient room temperature before use.

PRINCIPLE

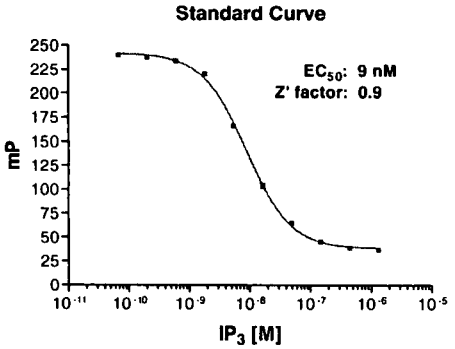
The HitHunter IP_3 FP is a rapid, homogeneous method for measuring cellular D-myo-Inositol 1,4,5-triphosphate. The assay is based on competitive binding between an IP_3 fluorescent tracer and unlabelled IP_3 from cell lysates or standards.

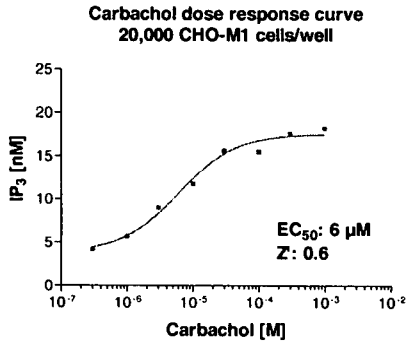
In the assay, induced cells are immediately quenched by perchloric acid. Fluorescent IP_3 tracer and the binding protein are subsequently added. IP_3 from cell lysates compete for binding to the IP_3 binding protein. Bound IP_3 tracer will "tumble" more slowly in solution, creating a polarized signal (high mP). The polarized signal is inversely proportional to the amount of IP_3 in the cell lysates.

INTENDED USE

This assay is used to monitor the levels of Inositol (1,4,5)-trisphosphate produced in cell lysates in a 384 well plate format using DiscoveRx Fluorescence Polarization technology.

REPRESENTATIVE DATA FOR HITHUNTER IP_3 FP ASSAY





[IP_3] is calculated from the standard curve

Technical Support: **Toll Free (866) 448-4864**
Phone (510) 979-1415 option 4

DiscoveRx

Product Insert 70-047 Rev. 1

HitHunter™ Inositol (1,4,5)-Trisphosphate Assay

Fluorescence Polarization Detection - Green
(HitHunter IP_3-FP Assay-Green)

Product Codes: 90-0037, 90-0037L

For Research Use in 96 or 384-well microtiter plates
Read the entire product insert fully before beginning the assay.

For additional information on DiscoveRx Technology or Technical Support, contact DiscoveRx or visit the website.

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KIT CONTENTS

HitHunter IP_3 FP Assay - green, contains:

Product Ordering Code		90-0037	90-0037L
# of 384-well test points		800	10,000
Item	Description	Volume	
1	IP_3 Standard - 20 µM	1.0 mL	10.0 mL
2	IP_3 Standard Dilution Buffer	10 mL	100 mL
3	Perchloric Acid (PCA) – 0.2 N	4 mL	50 mL
4	IP_3 Tracer - Green	8 mL	100 mL
5	IP_3 Binding Protein	16 mL	200 mL

MATERIALS NOT SUPPLIED

The following additional materials are required:

Equipment	Materials
• 384-well Black microtiter plates, non-binding, polypropylene; for optimal results use Greiner polypropylene non-binding assay plates (*Greiner product code 781209*) • Pipets and pipet tips • Fluorescence Polarization Reader and appropriate filters and dichroics. • Plate shaker	• HPLC grade water • PBS (without $CaCl_2$ and $MgCl_2$); *Sigma# D8537*

REAGENT PREPARATION

Thaw reagents completely and equilibrate to ambient room temperature before use.

IP_3 STANDARD - Dilute the IP_3 Standard (20 µM) to prepare (10) 3-fold serial dilutions in polypropylene tubes using the IP_3 Standard Dilution Buffer. For the zero standard, use the IP_3 Standard Dilution Buffer. The final system concentration of the standard curve ranges from 1.33×10^{-6} to 6.7×10^{-11} M IP_3.

PCA - Ready to use, do not mix with other reagents.

IP_3 TRACER - Ready to use, do not mix with other reagents.

IP_3 BINDING PROTEIN - Ready to use, do not mix with other reagents.

CELL PREPARATION - Harvest cells and resuspend in PBS (without $CaCl_2$ and $MgCl_2$) at the desired concentration. Titrate cells initially to find the optimum assay concentration for the specific cell line being tested. We recommend 5,000, 10,000, and 20,000 cells per well of a 384 plate as a starting point.

CONTROLS – Substitute water or PBS (without $CaCl_2$ and $MgCl_2$) for agonist/antagonist or standard as the zero control.

IMPORTANT NOTES FOR OPTIMAL PERFORMANCE

- Resuspend cells with an appropriate buffer that does not contain Calcium.
- It is critical to **add PCA 20 seconds following cell induction** in order to measure peak amounts of IP_3 in response to ligand-receptor activation.
- For manual pipetting, mixing on an automated plate shaker at 650 rpm for 5 minutes is recommended after step 4 and step 5 to reduce variability. This may not be necessary on automated pipetting systems where force of delivery provides sufficient mixing.

ASSAY PROCEDURE

It is recommended to assay each test point in triplicate. The following procedure is for 384-well format. For 96-well format multiply the volumes by a factor of 2.

Condition	Standard curve	Cells Agonist	Cells Antagonist
Step 1: Standard/ cells	10 µL standard	10 µL cells	10 µL cells
Step 2: Antagonist	-	-	5 µL antagonist
Incubate	-		30 minutes at 37°C**
Step 3: Agonist	5 µL water	5 µL agonist	5 µL agonist
Incubate	-	20 seconds (room temperature)**	
Step 4: PCA	5 µL PCA -0.2N		
Step 5: Tracer	10 µL IP_3 Tracer-Green		
Step 6: Binding Protein	20 µL IP_3 Binding Protein		
Gently tap plates for even mixing (shake plates for 5 minutes)			
Excitation Filter Fluorescein – 485 nm Emission Filter Fluorescein – 530 nm Dichroic Fluorescein – 505 nm			
Read Fluorescence Polarization signal. (Plates can be read immediately after step 6 or up to 16 hours later)			

**Note: The Incubation time for the antagonist or agonist may differ and should be optimized for each receptor type.

DATA ANALYSIS

Average the triplicate mP values for each test point. Plot the standard curve using a 4 parameter best-fit analysis.